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Genetic variants in *PDSS1* and *SLC16A6* in the ketone body metabolic pathway predict cutaneous melanoma-specific survival

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Abstract

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Author Contributions Statement

Wei Dai, Hongliang Liu, Ka Chen, Xinyuan Xu, and Danwen Qian mainly performed the data analysis process and wrote the manuscript. Chunying Li and Qingyi Wei supervised the work and wrote the manuscript. Sheng Luo, Christopher I. Amos, Jeffrey E. Lee, Xin Li and Hongmei Nan co-supervised the work and edited the manuscript. All authors edited and contributed to the final version of the manuscript.

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

A few single-nucleotide polymorphisms (SNPs) have been identified to be associated with cutaneous melanoma (CM) survival though genome-wide association studies, but stringent multiple testing corrections required for the hypothesis-free testing may have masked some true associations. Using a hypothesis-driven analysis approach, we sought to evaluate associations between SNPs in ketone body metabolic pathway genes and CM survival. We comprehensively assessed associations between 4,196 (538 genotyped and 3,658 imputed) common SNPs in ketone body metabolic pathway genes and CM survival, using a dataset of 858 patients of a case-control study from The University of Texas M.D. Anderson Cancer Center as the discovery set and another dataset of 409 patients from the Nurses' Health Study and the Health Professionals Follow-up Study as the replication set. There were 95/858 (11.1%) and 48/409 (11.5%) patients who died of CM, respectively. We identified two independent SNPs (i.e., *PDSS1* rs12254548 G>C and *SLC16A6* rs71387392 G>A) that were associated with CM survival, with allelic hazards ratios of 0.58 (95% confidence interval [CI]=0.44-0.76, $P=9.00\times10^{-5}$) and 1.98 (95% CI=1.34-2.94, $P=6.30\times10^{-4}$), respectively. Additionally, associations between genotypes of the SNPs and mRNA expression levels of their corresponding genes support the biologic plausibility of a role for these two variants in CM tumor progression and survival. Once validated by larger studies, *PDSS1* rs12254548 and *SLC16A6* rs71387392 may be biomarker for CM survival.

Keywords

cutaneous melanoma; ketone body metabolism; single-nucleotide polymorphism; genome-wide association study; cutaneous melanoma-specific survival

Introduction

Cutaneous melanoma (CM) is the most lethal human skin cancer, accounting for an estimated 96,480 new cases and 7,230 deaths in the United States in 2019 [1]. Though patients with a localized CM have a good prognosis, an advanced disease has a very low survival rate without aggressive immunotherapeutic treatment [2]. Despite advances in accurate prognosis for CM, a better understanding of genetic basis for the disease progression will help identify new prognostic and therapeutic biomarkers.

The importance of metabolic alterations in melanoma has been recognized [3]. For example, ketone bodies as an alternative source of metabolic energy, particularly during diminished carbohydrate availability, play important roles in metabolic signaling, post-translational modification, and inflammation regulation [4,5]. Connections between ketone body metabolism and cancer progression are accumulating increasingly, offering the possibility of precision-guided nutritional therapies [6]. Evidence suggests that the ketotic state enhances metabolic oxidative stress in cancer cells and thus influences cancer progression [7]. Although epidemiological associations between cancer and ketogenic diets are debatable, the regulation of cellular metabolism via ketogenic diets has been considered as an important co-adjuvant therapy in neurological disorders and cancer [8].

Recently, the aberrant expression of ketogenic enzymes have been reported in cancer cells of neuroectodermal origin, including melanoma [9]. Fenofibrate, a synthetic peroxisome proliferator-activated receptor alpha (PPARα) activator, induces beta-hydroxybutyrate

production and inhibits proliferation and metastasis in melanoma cells [10]. PPARα plays important roles in fatty acid oxidation and ketogenesis, and therefore fenofibrate might be useful as a complementary adjunct treatment of melanoma [11]. Specifically, 3-hydroxy-3-methylglutaryl-CoA lyase has been shown to induce intracellular accumulation of the ketone body acetoacetate, promoting mitogen-activated protein kinase/extracellular-signal regulated kinase signaling and growth in an oncogenic BRAF-dependent manner in melanoma cells [12]. Additionally, the ketone body acetoacetate selectively enhanced the tumor growth of BRAF V600E-expressing human melanoma cells in xenograft mice [13]. These observations raise the question of whether the ketone body metabolism plays a role in melanoma cell bioenergetics signaling.

Studies have shown that germline single-nucleotide polymorphisms (SNPs) are associated with cancer risk and survival [14,15], suggesting the importance of a genetic basis as a molecular mechanism underlying tumor progression. CM is no exception to this paradigm, and recent genome-wide association studies (GWASs) have demonstrated several SNPs to be associated with susceptibility to CM [16,17]. In addition, SNPs may modulate the growth characteristics of melanocytic cells [18,19]. Given the previous identification of SNPs associated with susceptibility to CM, it is likely that identifying genetic variants in additional signaling pathway genes will yield novel biomarkers for CM prognosis.

Considering the role of ketogenesis in melanoma growth, it is likely that genetic variants in the ketone body metabolic pathway genes could also serve as novel biomarkers of prognostic significance for CM patients. To test such a hypothesis, we performed a pathway-based multigene approach to identify SNPs in genes in the ketone body metabolic pathway and examined their associations with survival in CM patients using two published available GWAS datasets. It should also be noted that the typically highly stringent GWAS significance threshold could be much relaxed for such a targeted pathway-based approach, because the number of SNPs to be tested here is greatly reduced.

MATERIALS AND METHODS

Study populations

Patients with a GWAS dataset from The University of Texas MD Anderson Cancer Center (MDACC) were included in the discovery study, whereas patients with a GWAS dataset from the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) of Harvard University were included in the validation study. Detailed descriptions of subject selection and data collection for each study have been described elsewhere [16,20], and a written informed consent was obtained from all participants after approval by institutional review boards at both MD Anderson and Brigham and Women's Hospital. The MDACC and NHS/HPFS datasets included 858 and 409 non-Hispanic white patients with CM, respectively. For the MDACC GWAS study, genomic DNA extracted from the whole blood was genotyped using the Illumina HumanOmni-Quad_v1_0_B array and the National Center for Biotechnology Information Database of Genotypes and Phenotypes (dbGaP; http://www.ncbi.nlm.nih.gov/gap), accession number phs000187.v1.p1. Genome-wide imputation was then performed using the MACH software program based on the 1000 Genomes Project, phase I V2 CEU data (March 2010 release) [21]. For the NHS/HPFS GWAS

study, genotyping was performed using the Illumina HumanHap610 array. Genome-wide imputation was also conducted using the MACH software based on the 1000 Genomes Project CEU data (phase I v3, March 2012) [22,23].

Gene and SNP extraction

We selected 44 autosomal genes of the ketone body metabolism-related pathway according to the databases of Molecular Signatures Database and selected literature (Table S1). In brief, genotyped and imputed SNPs within 2-kb up- and down-stream of the genes were extracted from the MDACC GWAS dataset following the outlined quality control criteria: minor allele frequency (MAF) ≥ 0.05, genotyping success rate > 95%, and Hardy-Weinberg equilibrium *P* value > 1×10^{-5}, and from imputation for those SNPs with $r^2 \geq 0.8$. We performed linkage disequilibrium (LD) analysis using HaploView 4.2 according to 373 Europeans from the 1000 Genomes Project; pairwise $r^2 \geq 0.8$ was considered in a high LD.

In silico functional analysis

For those SNPs identified in the multivariate analysis as significant, we further performed bioinformatics functional prediction using online tools: SNPinfo (http://snpinfo.niehs.nih.gov), RegulomeDB (http://www.regulomedb.org) and HaploReg (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php). We then performed an expression quantitative trait loci (eQTL) analysis using several data sources: (1) lymphoblastoid cell data on 373 European individuals from the 1000 Genomes Project (phase I integrated release 3, March 2012, (2) the Genotype Tissue Expression (GTEx) project, and (3) primary cutaneous melanoma tissue data from The Cancer Genome Atlas (TCGA) database.

Statistical methods

For each GWAS dataset, we performed multivariate Cox proportional hazards regression analysis to evaluate associations between the SNPs and CMSS with adjustments of the available covariates, using the GenABEL package of R software. CMSS was determined by the time from diagnosis to last follow-up or the CM-related death time. In the MDACC study, tumor stages were divided into two groups: stages I/II and stages III/IV. Adjustments include age, sex, Breslow thickness, tumor stage, ulceration and mitotic rate for the MDACC dataset, but only age and sex for the NHS/HPFS dataset. The Bayesian false-discovery probability (BFDP) method with a cut-off value of 0.80 was used for multiple testing correction, because 87% of the SNPs were imputed and thus in a high LD with other SNPs [24]. The use of BFDP is statistically less stringent than false discovery rate, but it is more reasonable for the multiple testing correction for imputed SNPs in this gene-set analysis.

We also assigned a prior probability of 0.1 to detect a hazards ratio (HR) of 2.0 for an association with genotypes and alleles of each SNP in the two GWAS datasets. A meta-analysis was further performed to combine the results of two datasets. A fixed-effects model was used because no heterogeneity was found between the two datasets (the Cochran's Q test *P*-value > 0.100 and the heterogeneity statistic $I^2 < 50.0\%$). A Kaplan-Meier curve was used to estimate the HRs for CMSS-associated genotypes, and the combination of risk genotypes (those associated with increased risk of death) was also used to evaluate the

cumulative effects of selected SNPs. For stratified analyses, heterogeneity between subgroups was assessed with the χ^2-based Q-test and considered significant when $P < 0.05$. A time-dependent receiver operating characteristic (ROC) analysis was performed to calculate the area under the curve (AUC) for SNPs and clinical variables using the "timeROC" package of R software in the discovery dataset [25]. Furthermore, Haploview v4.231 and LocusZoom32 were used to construct Manhattan plots and regional association plots, respectively [26,27]. Correlations between SNPs and their mRNA expression was performed by using linear regression analysis [28]. Unless otherwise specified, all other statistical analyses were performed with SAS software (version 9.4; SAS Institute, Cary, NC).

RESULTS

Subject Characteristics

In the present study, we used a discovery dataset of 858 CM patients from MDACC and a validation dataset of 409 CM patients from NHS/HPFS (Table S2). In the MDACC dataset, there were slightly more male patients (496, 57.8%) than female patients, ranging in age between 17 and 94 years at diagnosis (52.4 ± 14.4 years); 56.8% of these cases were older than 50 years. Many more cases were diagnosed with stages I/II (709, 82.6%) than with stage III/IV (149, 17.4%). Median follow-up time (81.1 months) ranged between 4.7 and 175.3 months. In the NHS/HPFS dataset, the age range of CM patients was 34 and 87 years at diagnosis (61.1 ± 10.8 years); the majority of the cases were over 50 years old (337, 82.4%). There were nearly twice as many female patients (271, 66.3%) as male patients (138, 33.7%). The median follow-up time was 179.0 months (range 5.0 to 453.0 months). During follow-up 95/858 (11.1%) and 48/409 (11.5%) patients died of CM in the MDACC dataset and the NHS/HPFS dataset, respectively. We did not adjust for principal components in either the discovery or the validation dataset, because no principal components were significantly associated with CM survival, indicating the absence of any detectable population stratification in either the MDACC or the NHS/HPFS dataset.

Associations between SNPs in the ketone body metabolic pathway genes and CMSS

We present a flow chart of the study design in Figure 1. To assess the associations of 538 genotyped and 3,658 imputed SNPs of ketone body metabolic pathway genes with CMSS, we performed a single locus analysis in the MDACC dataset with adjustments for age, sex, tumor stage, Breslow thickness, ulceration, and mitotic rate. A Manhattan plot is provided in Figure S1; 211 SNPs were significantly associated with CMSS at $P < 0.05$, of which 196 SNPs were still significant after the multiple test correction by BFDP (Table S3). Next we assessed the associations between these 196 SNPs and CMSS in the 409 CM patients from the NHS/HPFS dataset, of which 173 SNPs were replicated. After Cox regression analysis with adjustment for age and sex, 14 SNPs (all imputed) of two genes were validated and considered significantly associated with CMSS at $P < 0.05$, including ten SNPs in *PDSS1* (decaprenyl diphosphate synthase subunit 1) and four SNPs in *SLC16A6* (solute carrier family 16 member 6) (Table 1). In the subsequent meta-analysis of these two studies, 14 SNPs in *PDSS1* and *SLC16A6* genes remained significant in associations with CMSS (Table 1), and no between-study heterogeneity was observed for these SNPs ($P_{het} > 0.05$ for both).

Genetic variants in the ketone body metabolic pathway genes as independent predictors

We further performed LD analysis of the 14 SNPs in *PDSS1* and *SLC16A6* and found that except for rs2368182, nine other SNPs (i.e., rs12254548, rs1809359, rs3808914, rs7896301, rs68159164, rs71483808, rs11015232, rs7904343 and rs1960383) in *PDSS1* were in high LD (Figure S2a). For *SLC16A6*, four SNPs were in high LD with each other (i.e., rs71387392, rs35924680, rs34080227 and rs12945324) (Figure S2b). Functional prediction indicated that five SNPs (i.e., rs1960383, rs12254548, rs3808914, rs7896301 and rs11015232) in *PDSS1* had a RegulomeDB scores < 4 and two SNPs in *SLC16A6* were suggested to be located in the 3'-UTR (Table S4). In consideration of *P* values, LD, and predicted functions, we selected rs2368182 and rs12254548 in *PDSS1* and rs71387392 in *SLC16A6* as the independent tagSNPs for further analysis. Then these three SNPs together with clinical prognostic variables were included in a multivariate stepwise Cox model from the MDACC dataset. As a result, *PDSS1* rs12254548 G>C and *SLC16A6* rs71387392 G>A remained in the model as independent predictors of CMSS (Table S5). For visual presentation, these two SNPs are shown in the regional association plots with an expansion of 50-kb in the flanks of the corresponding gene region, in which the two selected independent representative SNPs are labeled in purple. (Figure S3).

In the MDACC study (with adjustment for covariates where appropriate), a protective effect of the *PDSS1* rs12254548 C allele (P_{trend} = 0.005) but a risk effect of the *SLC16A6* rs71387392 A allele (P_{trend} = 0.006) on CM survival were statistically significant in the trend test. We also observed similar results for the *PDSS1* rs12254548 C allele in the NHS/HPFS dataset (P_{trend} = 0.004), and the combined dataset of MDACC and NHS/HPFS (P_{trend} < 0.0001) and for the *SLC16A6* rs71387392 A allele in the NHS/HPFS dataset (P_{trend} = 0.038) and the combined dataset of MDACC and NHS/HPFS (P_{trend} < 0.0001) (Table 2). We also present Kaplan-Meier survival curves of the associations with CMSS for risk genotypes of *PDSS1* rs12254548 and *SLC16A6* rs71387392 in Figure 2a-2f.

Survival of CM patients with combined risk genotypes

We combined the risk genotypes of *PDSS1* rs12254548 GG and *SLC16A6* rs71387392 GA +AA into one variable as a genetic score to estimate the joint effect of the two SNPs. We further categorized all the subjects into three groups: 0, 1, or 2 risk genotype. As shown in Table 2, we observed a risk-genotype dose-response effect on CMSS associated with the genetic score in the MDACC dataset (P_{trend} = 0.0001), the NHS/HPFS dataset (P_{trend} = 0.001), and the combined dataset of MDACC and NHS/HPFS (P_{trend} < 0.0001). After that, we dichotomized all subjects into the 0 risk genotype group and the 1-2 risk genotypes group because of the small number of subjects in some of the subgroups. Compared with the 0 risk genotype group, the 1-2 risk genotypes group had greater CM-death risk in the MDACC dataset (adjusted hazards ratio [HR_{adj}] = 2.18; 95% confidence interval [CI] = 1.39-3.41, *P* = 0.0007), the NHS/HPFS dataset (HR_{adj} = 2.84; 95% CI = 1.54-5.24, *P* = 0.0009) and the combined dataset of MDACC and NHS/HPFS (HR_{adj} = 2.50; 95% CI = 1.76-3.56, *P* < 0.0001). We also used Kaplan-Meier curves to illustrate the associations between the number of risk genotypes and CMSS (Figure 2g-2i).

Stratified analyses for the effect of combined risk genotypes on CMSS

Compared with the 0 risk genotype group, CM patients with 1-2 risk showed a substantially increased risk of CM-related death in the presence of clinical variables, which was evident in all the subgroups, except for those with tumor cell mitotic rate of ≤ 1/mm^2 in the MDACC dataset. However, no significant interaction was found among the subgroups (Table S6).

ROC curve and time-dependent AUC

In the ROC curve and time-dependent AUC, we further assessed the risk effect of the two independent SNPs in the presence of clinical variables where appropriate for improving the classification of 5-year CMSS in the MDACC dataset, the NHS/HPFS dataset and the combined dataset of both MDACC and NHS/HPFS. Consistently, the AUC of the five-year CMSS improved prediction performance in the above-mentioned three datasets (Figure S4a, 4c and 4e). The AUC of the 5-year CMSS increased from 85.71% to 86.26% (P = 0.528) with the addition of risk genotypes to the model, and this effect was not statistically significant in the MDACC dataset. But the AUC of the five-year CMSS in the NHS/HPFS dataset significantly increased from 54.05% to 68.76% (P = 0.003) with the addition of risk genotypes to the model. We also observed a borderline of P value equals to 0.050 in the combined dataset of both MDACC and NHS/HPFS. Through the entire follow-up period, we also used the time-dependent AUC curves to assess the ability of risk genotypes in CMSS prediction (Figure S4b, 4d and 4f).

Genotype-phenotype correlation analyses

To further explore the potential functions of these two tagSNPs, we performed eQTL to evaluate correlations between SNPs and mRNA expression levels in the 1000 Genomes Project [22,28]. Only the rs12254548 C allele demonstrated a significant association with an increased mRNA expression level of *PDSS1* in the additive model and the dominant model (P = 0.0006 and P = 0.0004, respectively, Figure 3a-3b), while this was not the case for the *SLC16A6* rs71387392 A allele (data not shown). However, in the TCGA data based on 59 samples of primary cutaneous melanoma tissue, the *SLC16A6* rs71387392 A allele was associated with an increased mRNA expression level of *SLC16A6* in a dominant model (P = 0.039, Figure S5). However, no significant associations were observed in the GTEx database. We also found the two SNPs (i.e., rs12254548 and rs71387392) to be located in a DNase I hypersensitive site, where CpG islands, and histone modification H3K27 acetylation may regulate activities of enhancer or promoter functions by using experimental data from the in ENCODE project. It has also been suggested that rs71387392 is located on the Hoxd8 motif by the DNase cluster and transcription factor CHIP-seq data (Figure S6).

Discussion

In the present study, we analyzed associations between SNPs in genes of the ketone body metabolism pathway and CMSS using two previously published datasets. We identified two SNPs (i.e., *PDSS1* rs12254548 and *SLC16A6* rs71387392) that were significantly associated with CMSS. In subsequent functional prediction analysis, we found that the *PDSS1* rs12254548 C allele was associated with increased mRNA expression levels of in the 373 established blood cell lines and that the *SLC16A6* rs71387392 A allele was associated

with increased mRNA expression levels in primary cutaneous melanoma tissues of 59 samples from the TCGA dataset.

Ketone bodies have been investigated in cancer cell biology via the fuel metabolism and a signaling mechanism [6]. Derangements of the ketone body metabolism can affect pathophysiological processes in cancer. Identification of such metabolic vulnerability has provided opportunities for prognostic and therapeutic strategies in cancer management [29]. For example, the overexpression of ketolytic enzymes has been reported as a prognostic biomarker associated with aggressive phenotypes in prostate cancer and colorectal cancer [30,31]. Venous blood ketone bodies could also predict prognosis of hepatocellular carcinoma after transcatheter arterial chemoembolization [32]. Importantly, there is also evidence that the β-hydroxybutyrate production, stimulated by the peroxisome proliferator-activated receptor α agonist fenofibrate, was associated with cell growth arrest and energy stress in murine melanoma cells, supporting the importance of the ketone body metabolism in melanoma progression [9].

PDSS1 is located on chromosome 10p12.1, encoding an enzyme that elongates the prenyl side-chain of ubiquinone, one of the elements in the respiratory chain, and some mutations in this gene have been reported. For example, a homozygous missense mutation in *PDSS1* leads to ubiquinone deficiency, which causes an early-onset hearing loss disorder with mitochondrial dysfunction [33]. Genome-wide gene expression studies have found that *PDSS1* expression levels were upregulated by exposing human osteosarcoma cells to bisphenol A analogs [34]. A pilot study also suggested that significant upregulation of PDSS1 expression levels in whole blood were associated with susceptibility to type 2 diabetes and therapeutic response [35]. Genetic variants in *PDSS1* have also been identified in patients with mitochondrial disorders, suggesting its potential role in mitochondrial function [36]. Because ketone bodies are involved in biological functions, the C allele of *PDSS1* rs12254548 is associated with increased mRNA expression levels but a better survival, suggesting that *PDSS1* is likely to be a tumor suppressor in CM progression and prognosis.

SLC16A6, located in chromosome 17q24.2, encodes monocarboxylate transporter 7 (MCT7), exporting β-hydroxybutyrate from the liver [37], which is important for the lipid metabolism. For example, a zebrafish model lacking SLC16A6 developed fatty liver during fasting, possibly due to the diversion of acetyl-CoA to lipid synthesis rather than to ketone bodies [38]. Although a pronounced MCT7 signal was observed in human muscle [39], the mechanistic activity of SLC16A6 has not been functionally elucidated. In addition, *SLC16A6* variants have been significantly associated with risk of breast cancer [40]. Another report has demonstrated that SLC16A6 expression was up-regulated in paclitaxel- and methotrexate- resistant human ovarian cancer cell lines [41]. Furthermore, microarray data also demonstrated that SLC16A6 expression levels were significantly upregulated in the melanoma cell lines exposed to nonsteroidal anti-inflammatory drugs, which suggests the possibility of patient selection in clinical settings [42].

The present study has some limitations. First, clinical information on the validation dataset from the NHS/HPFS study contains only age and sex, which might explain the difference in the AUCs between the two datasets we used. Second, neither of the two datasets had detailed

information on administration of a ketogenic diet, history of fatty liver disease, or systemic treatments and response, which should have been adjusted for the possible effect on patients' outcomes. Also, further functional investigations should be conducted to provide mechanistic insights into these two novel SNPs.

In conclusion, we report some significant associations between CMSS and genetic variants in *PDSS1* and *SLC16A6*. CM patients with more numerous risk variant genotypes had poorer survival. We believe that these results are likely biologically plausible, since the genotype-phenotype correlation demonstrates that *PDSS1* expression levels may be modulated by rs12254548, although additional investigation is needed to unravel the underlying molecular mechanisms. Our data allow us to better understand the role of ketone bodies in skin cancer biology and may open up new opportunities for their therapeutic application to CM clinical management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

CI	confidence interval
CM	cutaneous melanoma
CMSS	cutaneous melanoma-specific survival
GWAS	genome-wide association study
HR_{adj}	adjusted hazards ratio

LD	linkage disequilibrium
MDACC	The University of Texas MD Anderson Cancer Center
NHS	the Nurses' Health Study
HPFS	the Health Professionals Follow-up Study
PDSS1	decaprenyl diphosphate synthase subunit 1
SLC16A6	solute carrier family 16 member 6
SNP	single-nucleotide polymorphism

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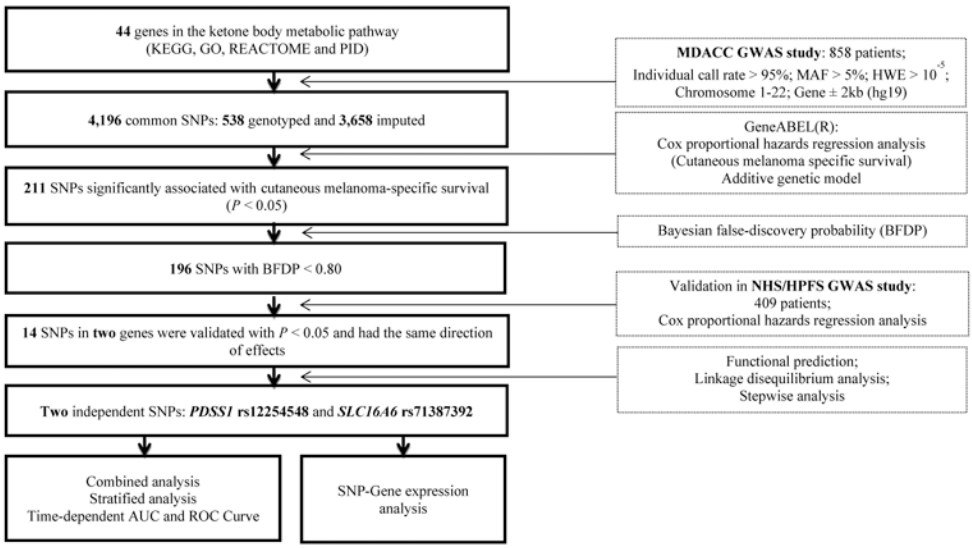

Figure 1. Research workflow for SNPs in the ketone body metabolic pathway genes.

Abbreviations: AUC, area under curve; BFDP, Bayesian false-discovery probability; CMSS, cutaneous melanoma-specific survival; GO, Gene Ontology; GWAS, genome wide association study; HWE, Hardy Weinberg equilibrium; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAF, minor allele frequency; MDACC, The University of Texas M.D. Anderson Cancer Center; NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; *PDSS1*, decaprenyl diphosphate synthase subunit 1; PID, Pathway Interaction Database; ROC, receiver operating characteristic; *SLC16A6*, solute carrier family 16 member 6; SNP, single nucleotide polymorphism.

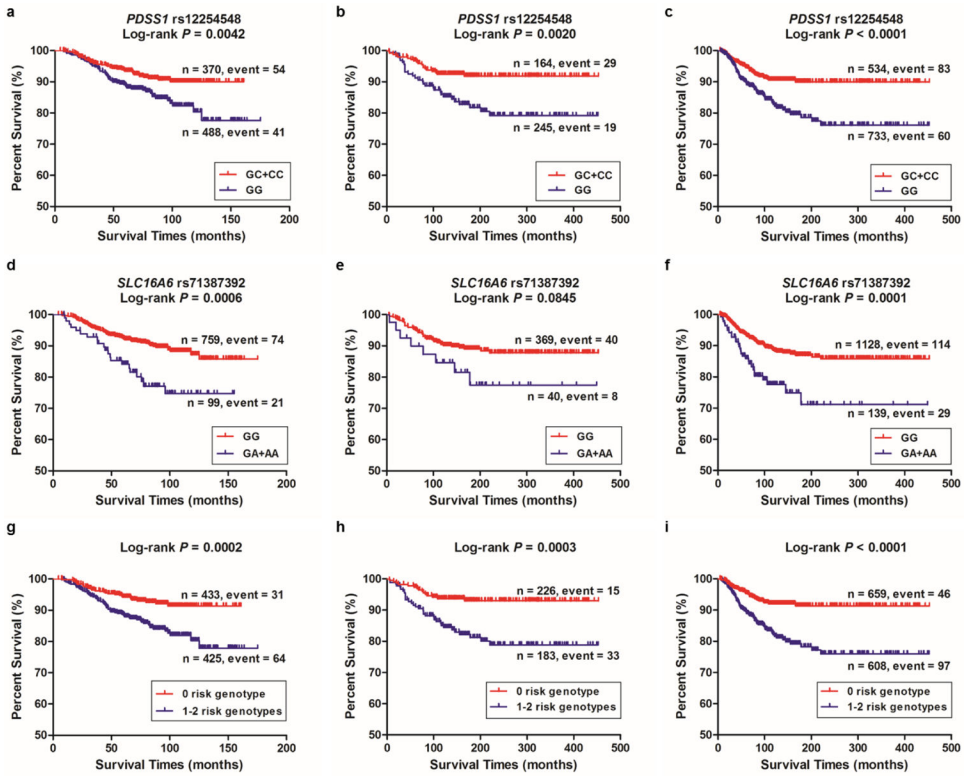

Figure 2. Selected SNPs and survival prediction.

Kaplan-Meier curves of cutaneous melanoma-specific survival (CMSS) stratified by *PDSS1* rs12254548, assuming a dominant model in (**a**) the MDACC, (**b**) the NHS/HPFS and (**c**) the MDACC and NHS/HPFS combined dataset. Kaplan-Meier curves of CMSS stratified by *SLC16A6* rs71387392 in (**d**) the MDACC, (**e**) the NHS/HPFS and (**f**) the MDACC and NHS/HPFS combined dataset. Kaplan-Meier survival curves of the combined risk genotypes on CMSS: dichotomized 0 risk genotype group and 1-2 risk genotypes group in (**g**) the MDACC, (**h**) the NHS/HPFS and (**i**) the MDACC and NHS/HPFS combined dataset. Abbreviations: SNP, single nucleotide polymorphism; CMSS, cutaneous melanoma-specific survival; MDACC, The University of Texas M.D. Anderson Cancer Center; NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; *PDSS1*, decaprenyl diphosphate synthase subunit 1; *SLC16A6*, solute carrier family 16 member 6.

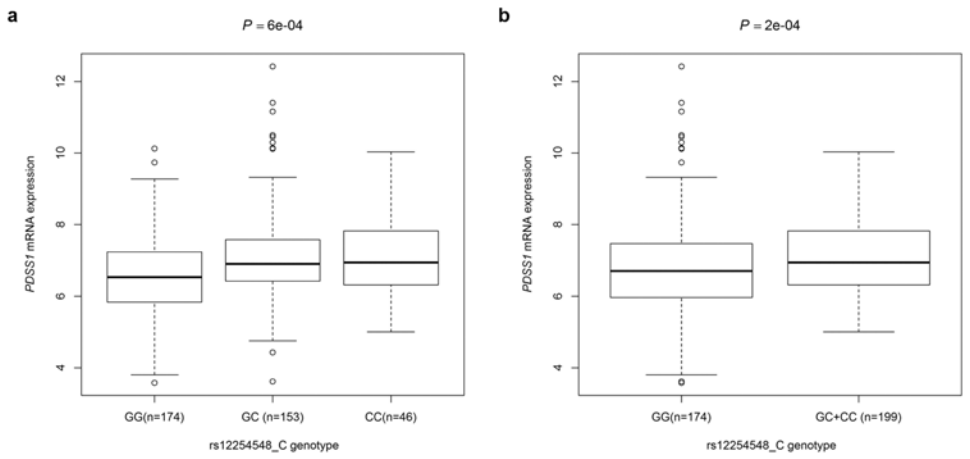

Figure 3. Associations between SNPs and mRNA expression levels of their corresponding genes.

The expression quantitative trait loci (eQTL) analysis from 373 European descendants from the 1000 Genomes Project for *PDSS1* rs12254548 in the additive model (**a**) and the dominant model (**b**). Abbreviations: *PDSS1*, decaprenyl diphosphate synthase subunit 1.

Table 1.

Meta-analysis of 14 validated SNPs in the ketone body metabolic pathway genes using two independently published melanoma GWAS datasets

SNP[1]	Allele[2]	Gene	Position	Discovery-MDACC (n=858)				Validation-NHS/HPFS (n=409)			Meta-analysis			
				EAF	HR (95% CI)[3]	*P*[3]	BFDP[4]	EAF	HR (95% CI)[5]	*P*[5]	P_{het}	I^2	HR (95% CI)[6]	*P*[6]
rs1809359	T>C	*PDSS1*	10p12.1	0.34	0.62 (0.44-0.86)	0.005	0.483	0.37	0.47 (0.29-0.77)	0.002	0.395	0	0.57 (0.43-0.75)	5.89×10^{-5}
rs12254548	G>C	*PDSS1*	10p12.1	0.34	0.62 (0.44-0.86)	0.005	0.425	0.37	0.50 (0.31-0.80)	0.004	0.468	0	0.58 (0.44-0.76)	9.00×10^{-5}
rs3808914	C>G	*PDSS1*	10p12.1	0.34	0.62 (0.44-0.86)	0.005	0.425	0.36	0.49 (0.31-0.81)	0.004	0.431	0	0.57 (0.44-0.76)	9.01×10^{-5}
rs7896301	A>T	*PDSS1*	10p12.1	0.34	0.67 (0.48-0.93)	0.015	0.669	0.37	0.50 (0.31-0.81)	0.004	0.325	0	0.61 (0.44-0.76)	3.41×10^{-4}
rs68159164	C>T	*PDSS1*	10p12.1	0.34	0.67 (0.48-0.93)	0.015	0.669	0.37	0.50 (0.31-0.81)	0.004	0.325	0	0.61 (0.47-0.80)	3.41×10^{-4}
rs71483808	A>G	*PDSS1*	10p12.1	0.34	0.67 (0.48-0.93)	0.015	0.669	0.37	0.50 (0.31-0.81)	0.004	0.325	0	0.61 (0.47-0.80)	3.41×10^{-4}
rs11015232	C>G	*PDSS1*	10p12.1	0.34	0.67 (0.48-0.93)	0.015	0.669	0.37	0.50 (0.31-0.81)	0.004	0.325	0	0.61 (0.47-0.80)	3.41×10^{-4}
rs7904343	T>C	*PDSS1*	10p12.1	0.34	0.67 (0.48-0.93)	0.015	0.669	0.37	0.50 (0.31-0.81)	0.004	0.325	0	0.61 (0.47-0.80)	3.50×10^{-4}
rs1960383	C>T	*PDSS1*	10p12.1	0.34	0.67 (0.48-0.93)	0.015	0.669	0.37	0.51 (0.32-0.82)	0.006	0.352	0	0.61 (0.47-0.80)	4.16×10^{-4}
rs2368182	G>C	*PDSS1*	10p12.1	0.24	0.61 (0.41-0.91)	0.014	0.666	0.25	0.42 (0.23-0.78)	0.005	0.316	1.66	0.55 (0.39-0.76)	3.41×10^{-4}
rs71387392	G>A	*SLC16A6*	17q24.2	0.06	1.93 (1.21-3.11)	0.006	0.572	0.05	2.11 (1.04-4.27)	0.038	0.837	0	1.98 (1.34-2.94)	6.30×10^{-4}
rs35924680	G>A	*SLC16A6*	17q24.2	0.06	1.93 (1.21-3.11)	0.006	0.572	0.05	2.11 (1.04-4.27)	0.038	0.837	0	1.98 (1.34-2.94)	6.30×10^{-4}
rs34080227	C>T	*SLC16A6*	17q24.2	0.06	1.93 (1.21-3.11)	0.006	0.572	0.05	2.11 (1.04-4.27)	0.038	0.837	0	1.98 (1.34-2.94)	6.30×10^{-4}
rs12945324	T>G	*SLC16A6*	17q24.2	0.06	1.93 (1.21-3.11)	0.006	0.512	0.05	2.11 (1.04-4.27)	0.038	0.837	0	1.98 (1.34-2.94)	6.30×10^{-4}

[1] These SNPs are all imputed SNPs in the MDACC dataset (imputation quality $r^2 > 0.8$);

[2] Reference allele/effect allele;

[3] Adjusted for age, sex, Breslow thickness, tumor stage, ulceration and mitotic rate in an additive genetic model;

[4] BFDP was used for multiple test correction with detected a highest hazard ratio of 2.0 and a prior probability of 0.1;

[5] Adjusted for age and sex in an additive genetic model;

[6] Meta-analysis in a fix-effects model.

Abbreviations: SNP, single-nucleotide polymorphism; GWAS, genome-wide association study; MDACC, The University of Texas MD Anderson Cancer Center; NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; EAF, effect allele frequency; HR, hazards ratio; CI, confidence interval; BFDP, Bayesian false-discovery probability; P_{het}, *P* value for heterogeneity by Cochrane's Q test; *PDSS1*, decaprenyl diphosphate synthase subunit 1; *SLC16A6*, solute carrier family 16 member 6.

Table 2.

Associations between two independent SNPs in the ketone body metabolic pathway genes and CMSS of patients in the MDACC dataset, the NHS/HPFS dataset and the combined dataset of MDACC and NHS/HPFS

Genotype	MDACC (n=858)				NHS/HPFS (n=409)				MDACC + NHS/HPFS (n=1267)			
	Frequency		Multivariate analysis[1]		Frequency		Multivariate analysis[2]		Frequency		Multivariate analysis[3]	
	All	Death (%)	HR (95% CI)	*P*	All	Death (%)	HR (95% CI)	*P*	All	Death (%)	HR (95% CI)	*P*
***PDSS1* rs12254548 G>C**												
GG	370	54 (14.59)	1.00		164	29 (17.68)	1.00		534	83 (15.54)	1.00	
GC	395	33 (8.35)	0.56 (0.36-0.88)	0.012	187	16 (8.56)	0.47 (0.25-0.86)	0.015	582	49 (8.42)	0.51 (0.36-0.73)	0.0002
CC	93	8 (8.60)	0.44 (0.20-0.95)	0.036	58	3 (5.17)	0.29 (0.09-0.94)	0.040	151	11 (7.28)	0.42 (0.22-0.78))	0.006
Trend test				0.005				0.004				<.0001
GC+CC	488	41 (8.40)	0.53 (0.35-0.82)	0.004	245	19 (7.76)	0.43 (0.24-0.76)	0.004	733	60 (8.19)	0.49 (0.35-0.68)	<.0001
***SLC16A6* rs71387392 G>A**												
GG	759	74 (9.75)	1.00		369	40 (10.84)	1.00		1128	114 (10.11)	1.00	
GA	96	20 (20.83)	1.86 (1.11-3.11)	0.018	39	7 (17.95)	1.70 (0.76-3.81)	0.195	135	27 (20.00)	2.17 (1.42-3.30)	0.0003
AA	3	1 (33.33)	5.44 (0.73-40.78)	0.100	1	1 (100.00)	18.46 (2.42-140.90)	0.005	4	2 (50.00)	4.14 (1.01-16.88)	0.048
Trend test				0.006				0.038				<.0001
GA+AA	99	21 (21.21)	1.93 (1.17-3.20)	0.010	40	8 (20.00)	1.92 (0.90-4.11)	0.094	139	29 (20.86)	2.24 (1.49-3.38)	0.0001
Combined number of risk genotypes[4]												
0	433	31 (7.16)	1.00		226	15 (6.64)	1.00		659	46 (6.98)	1.00	
1	381	53 (13.91)	2.02 (1.26-3.21)	0.003	162	29 (17.90)	2.81 (1.50-5.27)	0.001	543	82 (15.10)	2.36 (1.65-3.39)	<.0001
2	44	11 (25.00)	3.32 (1.65-6.71)	0.0008	21	4 (19.05)	3.03 (1.00-9.23)	0.051	65	15 (23.08)	3.69 (2.06-6.61)	<.0001
Trend test				0.0001				0.001				<.0001
0	433	31 (7.16)	1.00		226	15 (6.64)	1.00		659	46 (6.98)	1.00	
1-2	425	64 (15.06)	2.18 (1.39-3.41)	0.0007	183	33 (18.03)	2.84 (1.54-5.24)	0.0009	608	97 (15.95)	2.50 (1.76-3.56)	<.0001

[1] Adjusted for age, sex, Breslow thickness, tumor stage, ulceration and mitotic rate in the MDACC dataset;

[2] Adjusted for age and sex in the NHS/HPFS dataset;

[3] Adjusted for age and sex in the MDACC and NHS/HPFS combined dataset;

[4] Risk genotypes include *PDSS1* rs12254548 GG and *SLC16A6* rs71387392 GA+AA.

Abbreviations: SNP, single-nucleotide polymorphism; CMSS, cutaneous melanoma-specific survival; MDACC, The University of Texas MD Anderson Cancer Center; NHS, the Nurses' Health Study; HPFS, the Health Professionals Follow-up Study; HR, hazards ratio; CI, confidence interval; *PDSS1*, decaprenyl diphosphate synthase subunit 1; *SLC16A6*, solute carrier family 16 member 6.